

## Wishing Away Inflammation? New Links between Serotonin and TNF Signaling

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The idea that emotional states can influence health has been widely accepted in medicine since Roman times (1). This idea has been validated in the modern era; several neuroendocrine pathways have been elucidated that link neural and immune responses (2). These links function reciprocally: a number of neural signaling pathways trigger the release of immunomodulators, and cells of the nervous system sense inflammation through receptors for immune cytokines. The Hypothalamic-Pituitary-Adrenal Axis “translates” psychological stressors into glucocorticoid synthesis by the adrenal cortex and epinephrine synthesis by the adrenal medulla, leading to an immediate epinephrine-activated vasoactive response. Glucocorticoid-mediated immune modulation may provide the short-term advantage of suppressing inflammation during stress, whereas chronic endogenous production or exogenous administration of glucocorticoids results in immunosuppression. Both the benefits and detriments of these effects are seen in patients placed on glucocorticoid therapy for autoimmune and inflammatory diseases. A recent paper by Yu et al. identifies a novel pathway of crosstalk between neural and immune receptors with the observation that pharmacological agonists of 5-hydroxytryptamine<sub>2A</sub> (5-HT<sub>2A</sub>) receptors can block the pro-inflammatory effects of Tumor Necrosis Factor (TNF) on smooth muscle vascular cells (3).

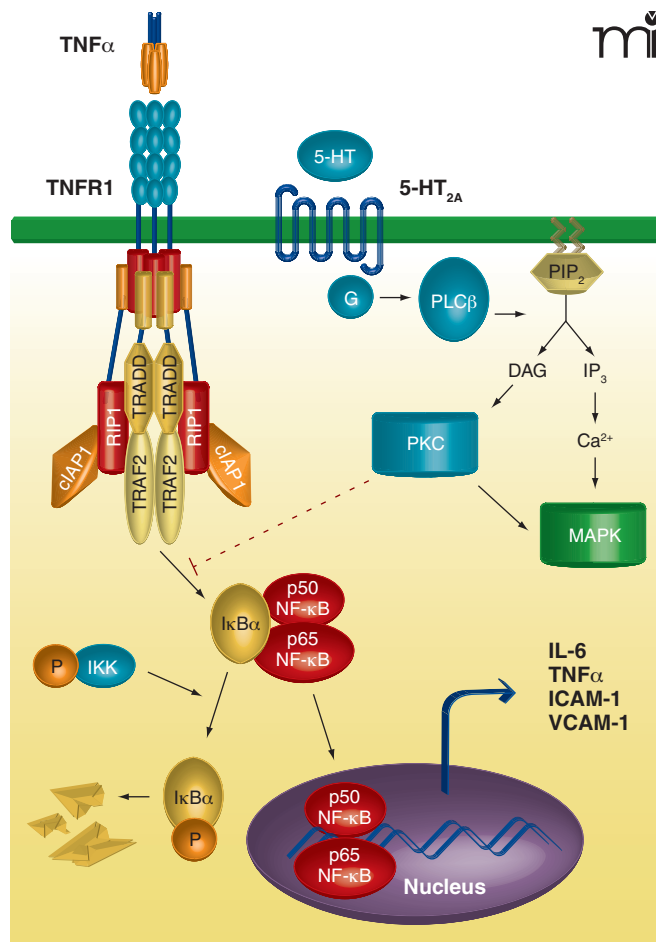
5-Hydroxytryptamine, commonly known as serotonin, is a neurotransmitter with potent effects on many aspects of mood and cognition. Selective serotonin reuptake inhibitors (SSRIs), see (4) are thought to mediate their antidepressant effects through enhancing the availability of serotonin in the CNS. Of the seven subtypes of serotonin receptors identified in humans, only the 5-HT<sub>2A</sub> receptor is involved in cognition. Hallucinogens such as lysergic acid diethylamide (LSD) are thought to exert their actions through 5-HT<sub>2A</sub> receptors, and atypical antipsychotics and some newer generation anti-depressants are thought to act primarily as 5-HT<sub>2A</sub> antagonists. For these reasons, the effects of serotonin on the CNS have been intensively studied. However, most serotonin is actually produced in other parts of the body by various cell types such as activated intestinal enterochromaffin cells, mast cells, and platelets. Serotonin regulates numerous biological processes outside the CNS such as bowel motility, platelet aggregation, cardiac function, and bladder control (5). In addition, serotonin has important effects on vascular and uterine smooth muscles, as it is involved in their growth and contraction (6). In the immune system, serotonin activates human monocytes and prevents their apoptosis (7) and modulates cytokine and chemokine production in lipopolysaccha-

ride (LPS)-primed monocytes (8). In vivo, serotonin appears to be pro-inflammatory, as a number of studies have shown depletion of serotonin within the CNS acts to reduce animal models of inflammation such as adjuvant-induced arthritis (9–11).

In contrast to the apparently pro-inflammatory role of serotonin as a neurotransmitter, Yu et al. (3) show that activation of 5-HT<sub>2A</sub> receptors on smooth muscle cells with the synthetic agonist (R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane [(R)-DOI] suppresses multiple responses to TNF. (R)-DOI repressed the TNF-mediated induction of adhesion molecules and cytokine production at concentrations in the low picomolar range, which might be considered “superpotent” because this is below the nanomolar affinity measured for (R)-DOI at 5-HT<sub>2A</sub> receptors. (R)-DOI suppressed TNF $\alpha$ -mediated induction of mRNA for proinflammatory markers, such as VCAM-1, ICAM-1, and IL-6, nitric oxide synthase activity, and translocation of NF- $\kappa$ B all at this very low concentration. These effects were restricted to 5-HT<sub>2A</sub> receptors, as 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptor-selective agonists were ineffective in suppressing TNF $\alpha$ -induced inflammation. (R)-DOI was also compared with three other 5-HT<sub>2A</sub> receptor agonists: the phenethylamine 2C-BCB [(4-bromo-3,6-dimethoxybenzocyclobuten-1-yl) methylamine]; and two indolealkylamines: LA-SS-Az [(2'S,4'S)-(+)-9,10-didehydro-6-methylergoline-8 $\beta$ -(trans-2,4-dimethylazetidide)] and LSD. These three agents suppressed TNF-induced ICAM-1, V-CAM-1, and IL-6 expression, but with less potency than (R)-DOI. In addition, (R)-DOI was able to significantly inhibit the effects of TNF $\alpha$  when administered up to four hours after TNF $\alpha$ , indicating possibly therapeutic effects of (R)-DOI on already established chronic inflammation.

Yu et al. (3) only hint at the signal transduction pathways underlying blockade of TNF signaling by the 5-HT<sub>2A</sub> agonists. TNF has two receptors, TNFR1 (p55) and TNFR2 (p75), but most of the pro-inflammatory effects of TNF are mediated through TNFR1, suggesting that 5-HT<sub>2A</sub> agonists likely act on TNFR1. Inhibitor studies suggested that the effects of 5-HT<sub>2A</sub> agonists are mediated through the activity of protein kinase C family members. TNFR1 signaling was affected by inhibition of NF- $\kappa$ B translocation to the nucleus, suggesting a direct effect of (R)-DOI on early events in TNFR1 signaling. The primary TNFR1 signaling complex consists of the adapter protein TNFR1-associated death domain protein (TRADD), the ring finger-containing proteins TNF receptor-associated factor 1 (TRAF1) and TRAF2, and the protein kinase receptor-interacting serine–threonine protein kinase (RIP), which act together to activate the I $\kappa$ B Kinase complex to trigger degradation of the inhibitor of NF- $\kappa$ B (I $\kappa$ B) and release of NF- $\kappa$ B subunits into the nucleus where they function as transcription factors (Figure 1). Interestingly, protein kinase C (PKC) agonists, such as Phorbol 12-Myristate 13-Acetate (PMA, also termed TPA) can block TNF signaling and inhibit assembly of the TNFR1 proximal signaling complex (12).

Although these results suggest that 5-HT<sub>2A</sub> agonists might be investigated further as an anti-inflammatory agent, there are a



**Figure 1. Potential mechanism of crosstalk between the tumor necrosis factor receptor 1 (TNFR1) and serotonin subtype 2A (5-HT<sub>2A</sub>) receptor signaling.** The primary TNFR1 signaling complex consists of the adapter protein TRADD, the ring finger-containing protein TRAF2, the inhibitor of apoptosis protein cIAP1, and the protein kinase RIP1. Following TNF binding, the receptor complex activates the IκB kinase complex to trigger IκB degradation and the release of NF-κB subunits into the nucleus where they function as transcription factors. The TNF-bound receptor complex also induces the expression of several pro-inflammatory genes such as IL-6, TNFα, ICAM-1, and VCAM-1. The receptor for serotonin, 5-HT<sub>2A</sub> is a G protein-coupled receptor able to stimulate phospholipase C (PLC), a membrane-bound enzyme that catalyzes the degradation of the inositol lipid phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>), producing inositol 1,4,5-trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). IP<sub>3</sub> mobilizes Ca<sup>2+</sup> that induces multiple responses in the cell, including activation of mitogen-activated protein kinase (MAPK), while DAG activates another kinase family, protein kinase C (PKC). Results from Yu and colleagues (3) suggest that 5-HT<sub>2A</sub> receptor-mediated anti-inflammatory effects are mediated through activation of PKC, which acts most probably at a level proximal to NF-κB nuclear translocation. TRADD, TNFR1-associated death domain protein; TRAF2, TNF receptor-associated factor 2; cIAP1, cellular inhibitor of apoptosis 1; RIP, receptor-interacting serine-threonine protein kinase; IL-6, interleukin-6; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

number of concerns that limit interpretation of these results. The suppressive effects of (R)-DOI on induction of pro-inflammatory genes were only reported for mRNAs. It is certainly necessary to determine whether these effects operate at the protein level as well. Most experiments were done with (R)-DOI, or other synthetic 5-HT<sub>2A</sub> receptor agonists. It would be useful to know whether these effects are seen with serotonin itself and whether they could be reversed by a 5-HT<sub>2A</sub> receptor antagonist such as ketanserin or by using cells deficient in the 5-HT<sub>2A</sub> receptor. Additionally, it is essential to test whether the observed effects of (R)-DOI on TNFα-induced inflammation also apply to other cell types, especially primary human cells responsive to TNF such as hepatocytes, endothelial cells, and monocytes.

Despite these concerns, it is tempting to speculate on the clinical implications of this study. Single-nucleotide genetic polymorphisms in the 5-HT<sub>2A</sub> receptor gene have been associated with rheumatoid arthritis (13), although the genome-wide significance of this association is not clear. Although not tested in this study, one might predict from these results that patients on 5-HT<sub>2A</sub>-blocking agents would become hypersensitive to the effects of TNF and possibly develop arthritic symptoms. In fact, in a recent retrospective survey of drug reactions, Dahlqvist and colleagues found a 45-fold excess rate of joint complaints in patients given 5-HT<sub>2A</sub>-blocking antidepressants, such as mianserin, nefazodone, and mirtazapin compared to patients given SSRIs (14). The same group observed that the density of serotonin 5-HT<sub>2A</sub> receptors was decreased in rheumatoid arthritis patients (15), suggesting a possible role for reduced expression of the 5-HT<sub>2A</sub> receptor in the sensitization of cells to TNF in rheumatoid arthritis. One might also speculate that SSRIs could be anti-inflammatory, although there is little evidence of this in the literature, and SSRIs would increase availability of serotonin for all subgroups of receptors, not just 5-HT<sub>2A</sub>.

Stimulating 5-HT<sub>2A</sub> receptors to suppress inflammation would clearly require the development of agents that do not affect the CNS, as 5-HT<sub>2A</sub> agonists such as LSD are well known to induce hallucinations and altered mental status. Also, the discovery that (R)-DOI was able to potently inhibit the effects of TNFα even for up to two hours after the administration of TNFα indicate that potential therapies could not only be aimed at preventing inflammation but also treating inflammatory injury that has already occurred or is ongoing. However, it would be important to determine the duration of the effects and whether receptor desensitization occurs, because treatment with 5-HT<sub>2A</sub> agonists would most likely be needed for much longer periods of time in patients with chronic inflammatory joint disease. Whether these results bear fruit in terms of therapeutic agents, they add one more thread to the intriguing web of interconnections that link our immune and nervous systems.

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